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| <b>(54) Title:</b> INCORPORATION OF TAXOL INTO LIPOSOMES AND GELS<br><br><b>(57) Abstract</b><br><br>Provided herein are liposomal or lipid-based gel formulations of taxol and a lipid containing a ratio of taxol to lipid (weight/weight) of from about 0.01:1 to at least about 0.10:1. These formulations are useful for treating animals afflicted with cancers.   |           |  |

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### INCORPORATION OF TAXOL INTO LIPOSOMES AND GELS

5 Taxol is a product of the pacific yew Taxus brevifolia. The drug (C<sub>47</sub>H<sub>51</sub>NO<sub>14</sub>, Tax-11-en-9-one), a member of a family of compounds comprising a taxane ring, is largely insoluble in aqueous solutions (see, e.g., Wani et al., J. Am. Chem. Soc. 43(9): 2325 (1971); NCI Investigational Drugs, NIH Publication No. 88-2654, August, 1988, pp. 94-97; Suffness and Cordell,  
10 "Antitumor Alkaloids," in: The Alkaloids, Volume XXV, Academic Press, New York, pp. 5-18 (1985)).

Taxol is a potent antimitotic agent which acts by binding to tubulin dimers and promoting their assembly into microtubules (see, e.g., Schiff et al.,  
15 Nature 277: 665 (1979); Schiff and Horwitz, Proc. Natl. Acad. Sci. USA 77(3):1561 (1980)). The bound drug stabilizes the microtubules and inhibits their disassembly, thereby inhibiting further cell division. Drug action, which is optimal at about equimolar concentrations of taxol and tubulin, may be inhibited by colchicine or podophyllotoxin (see, e.g., Suffness and Cordell, *supra*).

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This antimitotic activity, and its ability to inhibit cell migration, make taxol a valuable anticancer agent. Taxol's activity against ovarian, lung, brain, breast and other types of tumors has been well documented (see, e.g., Borman, Chemical and Engineering News, September 2, 1991; Edgerton, Biotechnology,  
25 9: 933 (1991); The Pharmacological Basis of Therapeutics (Goodman Gilman et al., eds.), Pergamon Press, New York (1990); Rizzo et al., J. Pharm. & Biomed. Anal. 8(2):159 (1990); Donehower et al., Cancer Treat. Rep. 71(12):1171 (1987) Grem et al., Cancer Treat. Rep. 71(12):1179; Wiernik et al., J. Clin. Oncol. 5(8):1232 (1987)).

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Taxol is largely insoluble in aqueous solutions. The drug is currently supplied as a 6 mg/ml suspension in CremophorEL™ (Bristol-Myers Squibb), a 50:50 mixture of Cremophor, a polyoxyethylated derivative of castor oil, and ethanol. The suspension is diluted to 0.6 mg/ml taxol for clinical use. However,  
35 administration of this formulation entails premedication with other drugs and a slow infusion of a large volume over 24 hours, to avoid toxicity associated with

the Cremophor vehicle. This administration procedure requires that patients receiving taxol be admitted to hospitals overnight. Additionally, because of the poor solubility of taxol in aqueous solutions, care must be exercised that the taxol does not precipitate from solution. Furthermore, taxol administration is frequently accompanied by myelosuppression and sensory neuropathy, and may also be accompanied by leukopenia and thrombocytopenia (see, e.g., Wiernik et al., *supra*; Donehower et al., *supra*; Grem et al., *supra*). Accordingly, there is a need for a carrier which can deliver taxol in a therapeutically useful, less toxic form.

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The liposomes and lipid-based gel formulations of taxol provided herein fill this need. Liposomes are spontaneously self-assembling structures comprising one or more bilayers of amphipathic lipid molecules enclosing an internal aqueous volume. The amphipathic lipid molecules which make up lipid bilayers comprise a polar (hydrophilic) headgroup region covalently linked to one or more non-polar (hydrophobic) acyl chains. The energetically unfavorable contact between the hydrophobic acyl chains and the aqueous medium causes the molecules to rearrange such that the polar headgroups are facing the aqueous medium internal or external to the liposome, while the acyl chains reorient towards the bilayer interior. The net result is an energetically stable structure in which the acyl chains are effectively shielded from coming into contact with the aqueous medium.

A variety of methods exist for producing liposomes (for a review, see, e.g., Szoka and Paphadjopoulos, in: Liposomes: From Physical Structure to Therapeutic Applications (C.G. Knight, ed., Elsevier/North Holland, pp. 51-82 (1981); Cullis et al., in: Liposomes. From Biophysics to Therapeutics M. J. Ostro, ed.), Marcel Dekker, pp. 39-72 (1987)). Bangham's original preparation (J. Mol. Biol. 13:238 (1965)) involves suspending phospholipids in an organic solution and then evaporating the solution to dryness, leaving a phospholipid film on the walls of the reaction vessel. Next, an appropriate amount of a chosen aqueous medium is added; the resulting liposomes, which consist of multilamellar vesicles (MLVs), are dispersed by mechanical means. This technique provided the basis for the development of sonicated unilamellar vesicles by Paphadjopoulos et al. (Biochem. Biophys. Acta. 135:624 (1968)). Lenk et al. (U.S. Patent Nos. 4,522,803, 5,030,453 and 5,169,637) and Fountain et al. (U.S.

Patent No. 4,588,708) disclose methods for producing multilamellar liposomes with substantially equal interlamellar solute distribution. FATMLVs, freeze-and-thaw multilamellar vesicles, also have substantially equal interlamellar solute distribution (Cullis et al., U.S. Patent No. 4,975,282). These vesicles are produced by first dispersing a lipid in an aqueous solvent to form multilamellar liposomes. The resulting lipid vesicles are rapidly frozen, the frozen mixture is warmed, and then the freeze-thaw cycle is repeated at least three times. Furthermore, Janoff et al. (U.S. Patent No. 4,721,612) and Bolcsak et al. (U.S. Patent No. 5,100,662) describe the preparation of liposomes of enhanced stability using sterols. Cullis et al. (U.S. Patent No. 5,008,050) and Loughrey et al. (U.S. Patent No. 5,059,421) disclose the preparation of a population of liposomes with a defined size distribution by extrusion of liposomes through filters under pressure. The disclosures of these publications are incorporated herein by reference.

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Bioactive molecules entrapped within liposomes can have an enhanced therapeutic index and improved biodistribution. Liposomal drugs are gradually released in the circulation, thereby alleviating the toxic side effects associated with administration of the free drug and minimizing the amount of the drug that need be administered to maintain desired serum levels. Additionally, drug-lipid formulations may be directed to intracellular sites of infection.

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#### Summary of the Invention

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This invention provides a liposome having a bilayer which comprises taxol and a lipid comprising a lysophospholipid, wherein the ratio of taxol to lipid (w/w) is from about 0.01:1 to at least about 0.10:1. In one embodiment of the invention, the liposome is a unilamellar liposome, preferably a large unilamellar liposome. In another embodiment of the invention, the liposome comprises a plurality of lipid bilayers each of which encloses an aqueous compartment, i.e., the liposome is a multilamellar vesicle. Preferably, this is a liposome wherein each of the aqueous compartments comprises a solute and the concentration of the solute in each of the aqueous compartments is substantially equal. The lysophospholipid is preferably a lysophosphatidylethanolamine, most preferably monooleoyl phosphatidylethanolamine (MOPE). The lipid can further

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comprise a sterol, preferably cholesterol.

This invention also provides a process of preparing a liposome having a bilayer which comprises taxol and a lipid comprising a lysophospholipid, which comprises the steps of: mixing taxol and the lipid; and, contacting the resulting  
5 mixture with an aqueous medium having a pH effective to form a liposome, wherein the aqueous medium is both internal and external to the liposome, and wherein the ratio of taxol to lipid (w/w) in the liposome is from about 0.01:1 to at least about 0.10:1. The lysophospholipid is preferably a  
10 lysophosphatidylethanolamine, most preferably monooleoyl phosphatidylethanolamine. The lipid can further comprise a sterol, preferably cholesterol. This invention provides the liposome prepared by this process. The process may comprise the additional step of increasing the pH of the aqueous medium external to the liposome to a pH effective to form a gel. The invention will then also provide the gel formed by this process..

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This invention further provides a process of preparing a gel comprising taxol and a lipid comprising a lysophospholipid which comprises the steps of: mixing taxol and the lipid; and, contacting the resulting mixture with an aqueous medium having a pH effective to form a gel, wherein the ratio of taxol  
20 to lipid (w/w) in the gel is from about 0.01 to at least about 0.10. The lysophospholipid is preferably a lysophosphatidylethanolamine, most preferably monooleoyl phosphatidylethanolamine. The lipid can further comprise a sterol, preferably cholesterol. This invention provides the gel prepared by this process. The process may comprise the additional step of decreasing the pH of the  
25 aqueous medium surrounding the gel to a pH effective to form liposomes. Decreasing the pH of the surrounding aqueous medium to an effective liposome forming pH may be accomplished by administering the gel to the circulatory system of an animal. That is, the liposomes may actually be formed in the animal that is the subject of treatment. The invention will then also provide the  
30 liposomes formed by this process.

Still further provided herein is a process of preparing a liposome comprising taxol and a lipid which comprises the steps of: dispersing a mixture comprising taxol and the lipid in an organic solvent; contacting the resulting  
35 dispersion with an aqueous medium; concurrently emulsifying the aqueous medium while evaporating the organic solvent, so as to form a liposome

comprising a plurality of lipid bilayers each of which encloses an aqueous compartment, wherein each of the aqueous compartments comprises a solute and wherein the concentration of the solute in each of the aqueous compartments is substantially equal; and, passing the liposome under pressure  
5 through a filter comprising pores of a defined size so as to reduce the lamellarity of the liposome, wherein the ratio of taxol to lipid (w/w) in the liposome is from about 0.01: to at least about 0.10:1. Preferably the liposome is a unilamellar liposome. The filters used to produce unilamellar liposomes preferably have pore sizes of about 100 nm or about 200 nm. The invention provides the  
10 liposome prepared by this process. This liposome, as well as the other liposomes provided herein, may be dehydrated and stored stably over an extended period of time.

This invention provides a pharmaceutical composition comprising a  
15 pharmaceutically acceptable carrier and a liposome comprising taxol is provided herein. Pharmaceutical compositions comprising the liposomes of this invention can be administered, in amounts effective to treat a cancer, to animals afflicted with the cancer, e.g., ovarian, brain, colon, lung or breast cancer. Presently, it is preferred that the cancer being treated is an ovarian cancer. Typically, the  
20 animal being treated is a mammal, desirably, a human. Furthermore, this invention provides a unit dosage form of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective anticancer amount of a liposome provided herein. Preferably, the effective anticancer amount of the liposome is from about 0.1 mg of the liposome per kg of body  
25 weight of the animal being treated to about 100 mg per kg of body weight.

Further provided is a method of treating an animal afflicted with a cancer which comprises administering to the animal an amount of a gel comprising taxol and a lipid provided herein effective to treat the cancer. The  
30 gel may be provided in bulk or as a unit dosage form comprising an amount of the gel effective to treat a cancer in the animal.

#### Brief Description of the Drawings

35 Figure 1. Gaussian analysis of the size distribution of EPC multilamellar liposomes having substantially equal interlamellar solute distribution (no taxol).

Run time: 5 min, 39 sec; average count rate: 340.3 kHz; channel width: 21.0  $\mu$  sec; temp.: 23 deg. C.; viscosity: 0.9325 centipoise; index of refraction: 1.333; print AT data: 500 kcounts; number of printouts: 10. Mean diameter: 204.5 nm; standard deviation: 75.1 nm (26.8%); chi squared: 0.2; baseline adjust: 0.00%;  
5 data: 508.2k.

Figure 2. Gaussian analysis of the size distribution of EPC SPLVs containing taxol. Run time: 6 min, 25 sec; average count rate: 345.5 kHz; channel width: 26.0  $\mu$ sec; temp.: 23 deg. C.; viscosity: 0.9325 centipoise; index of refraction:  
10 1.333; print AT data: 500 kcounts; number of printouts: 10. Mean diameter: 209.8 nm; standard deviation: 187.2 nm (60.4%); chi squared: 4.8; baseline adjust: 0.01%; data: 698.4k.

Figure 3. Gaussian analysis of the size distribution of EPC/taxol unilamellar  
15 liposomes produced by extrusion of SPLVs through polycarbonate filters with 0.1 micron pores (LUVET 100s). Run time: 8 min, 42 sec; average count rate: 356.5 kHz; channel width: 12.0  $\mu$ sec; temp.: 23 deg. C.; viscosity: 0.9325 centipoise; index of refraction: 1.333; print AT data: 500 kcounts; number of printouts: 10. Mean diameter: 105.2 nm; standard deviation: 22.3 nm (21.2%); chi squared:  
20 0.3; baseline adjust: 0.08%; data: 503.6k.

Figure 4. Gaussian analysis of the size distribution of EPC/taxol unilamellar liposomes produced by extrusion of SPLVs through polycarbonate filters with 0.2 micron pores (LUVET 200s). Run time: 8 min, 42 sec; average count rate: 345.9  
25 kHz; channel width: 16.0  $\mu$ sec; temp.: 23 deg. C.; viscosity: 0.9325 centipoise; index of refraction: 1.333; print AT data: 500 kcounts; number of printouts: 10. Mean diameter: 160.8 nm; standard deviation: 47.0 nm (29.2%); chi squared: 0.3; baseline adjust: 0.00%; data: 624.8k.

### 30 Detailed Description of the Invention

This invention provides a liposome having a bilayer which comprises taxol and a lipid comprising a lysophospholipid, wherein the ratio of taxol to lipid (w/w) is typically from about 0.01:1 to about 0.10:1, desirably about 0.03:1.  
35 However, the ratio may be higher or lower as needed. The lower limits will be governed by the least amount of taxol it is practical to make liposomes with, and



may be readily determined by ordinarily skilled artisans. The upper limits will be governed by the concentration at which taxol crystallizes, i.e., the concentration at which it separates out from lipid bilayers and forms aggregates. "Taxol" as used herein is meant to include the compound itself (C<sub>47</sub>H<sub>51</sub>NO<sub>14</sub>, tax-11-en-9-one) as well as taxol analogs, i.e., those compounds, whether synthetically produced or naturally derived, with similar structures and activities. Taxotère, which differs from taxol by having a *tert*-butoxycarbonyl group instead of a benzoyl group on the C-13 side chain, and a hydroxyl group instead of an acetoxyl group at C-10, is one such analog (see, e.g., Borman, *supra*). Other analogs include those which are derivatives of a hydroxylated taxane compound found in yew needles, e.g., baccatin-based 14-hydroxy taxol analogs such as 14-hydroxy-10-deacetylbaccatin III (see, e.g., Borman, Chemical and Engineering News, April 12, 1993, pp. 36-37).

"Liposomes" are spontaneously self-assembling structures comprising one or more bilayers of amphipathic lipid molecules, each of which encloses an internal aqueous volume. The amphipathic lipid molecules which make up lipid bilayers comprise a polar (hydrophilic) headgroup region covalently linked to one or more non-polar (hydrophobic) acyl chains. The energetically unfavorable contact between the hydrophobic acyl chains and the aqueous medium causes the molecules to rearrange such that the polar headgroups are facing the aqueous medium while the acyl chains reorient towards the interior of the bilayer. The net result is an energetically stable structure in which the acyl chains are effectively shielded from coming into contact with the aqueous medium.

In one embodiment of this invention, the liposome is a unilamellar liposome, preferably a large unilamellar liposome, i.e., a liposome with one lipid bilayer per vesicle having a diameter of at least 50 nm. More preferably, the unilamellar liposome is a LUVET<sub>100</sub> or LUVET<sub>200</sub>, i.e., a unilamellar liposome produced by extrusion of multilamellar liposome through filters with pore sizes of 100 nm or 200 nm, respectively (see Cullis et al., U.S. Patent No. 5,008,050 and Loughrey et al., U.S. Patent No. 5,059,421, the contents of which are incorporated herein by reference). Alternatively, the liposome comprises a plurality of lipid bilayers each of which encloses an aqueous compartment. Preferably, the liposome is one wherein each of the aqueous compartments

comprises a solute and wherein the concentration of the solute in each of the aqueous compartments is substantially equal, i.e., the liposome has substantially equal interlamellar solute distribution. The lipid used to prepare these liposomes comprises a lysophospholipid, i.e., a lipid with a phosphatidyl  
5 headgroup and a single acyl chain. The presently preferred lysophospholipids are lysophosphatidyl ethanolamines, most preferably, monooleoyl phosphatidylethanolamine (MOPE). The lipid may further comprise additional substituents, selected according to the need to prepare liposomes with specific additional properties. Such substituents, and the properties they confer on lipid  
10 vesicles, are well known to those of ordinary skill in the art. For example, the lipid may further comprise a sterol, which is typically incorporated in bilayers to increase their stability. Preferably, this sterol is cholesterol.

This invention also provides a process of preparing a liposome having a  
15 bilayer which comprises taxol and a lipid comprising a lysophospholipid, which comprises the steps of: mixing taxol and the lipid; and, contacting the resulting mixture with an aqueous medium having a pH effective to form a liposome, wherein the aqueous medium is both internal and external to the liposome, and wherein the ratio of taxol to lipid (w/w) in the liposome is typically from about  
20 0.01:1 to about 0.10:1, desirably about 0.03:1. However, the ratio may be higher or lower as needed. The lower limits will be governed by the least amount of taxol it is practical to make liposomes with, and may be readily determined by ordinarily skilled artisans. The upper limits will be governed by the concentration at which taxol crystallizes, i.e., the concentration at which it  
25 separates out from lipid bilayers and forms aggregates. This amount may also readily be determined by ordinarily skilled artisans given the knowledge provided herein. The lipid used to prepare these liposomes comprises a lysophospholipid, i.e., a lipid with a phosphatidyl headgroup and a single acyl chain. The presently preferred lysophospholipids are  
30 lysophosphatidylethanolamines, most preferably, monooleoyl phosphatidylethanolamine (MOPE). When the lipid is MOPE, the pH effective to form liposomes is preferably a pH of about 7.5.

The lipid may further comprise additional substituents, selected  
35 according to the need to prepare liposomes with specific additional properties. Such substituents, and the properties they confer on lipid vesicles, are well

known to those of ordinary skill in the art. For example, the lipid may further comprise a sterol, which is typically incorporated into lipid bilayers to enhance their stability (see Janoff et al., U.S. Patent No. 4,721,612 and Bolcsak et al., U.S. Patent No. 5,100,662). Preferably, this sterol is cholesterol. The liposome  
5 prepared by this process is also provided. The process may comprise the additional step of increasing the pH of the aqueous medium external to the liposome to a pH effective to form a gel. When the lipid comprises MOPE, the pH effective to form a gel is preferably a pH of about 9. The gel formed by such a process is also provided.

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Disclosed and claimed herein is a process of preparing a gel which comprises taxol and a lipid comprising a lysophospholipid, which comprises the steps of: mixing taxol and the lipid; and, contacting the resulting mixture with an aqueous medium having a pH effective to form a gel, wherein the ratio of  
15 taxol to lipid (w/w) in the gel is typically from about 0.01:1 to about 0.10:1, desirably about 0.03:1. However, the ratio may be higher or lower as needed. The lower limits will be governed by the least amount of taxol it is practical to make liposomes with, which may be readily determined by ordinarily skilled artisans. The upper limits will be governed by the concentration at which taxol  
20 crystallizes, i.e., the concentration at which it separates out from lipid bilayers and forms aggregates. The lipid used to prepare these liposomes comprises a lysophospholipid, i.e., a lipid with a phosphatidyl headgroup and a single acyl chain. The presently preferred lysophospholipids are lysophosphatidylethanolamines, most preferably, monooleoyl  
25 phosphatidylethanolamine (MOPE). The lipid may further comprise additional substituents, selected according to the need to prepare gels with specific additional properties. Such substituents, and the properties they confer on lipid vesicles, are well known to those of ordinary skill in the art. For example, the lipid may further comprise a sterol, preferably, cholesterol, which is typically  
30 incorporated into lipid bilayers to enhance their stability. Gels prepared by this process are also provided.

The process may further comprise decreasing the pH of the aqueous medium surrounding the gel so that the aqueous medium has a pH effective to  
35 form a liposome. When the lipid used comprises MOPE, the pH effective to form a liposome is preferably a pH of about 7.5. Liposomes prepared by such a

process are also provided herein. Decreasing the pH of the aqueous medium surrounding the gel may be accomplished by administering the gel to the circulatory system of the animal that is the subject of treatment with taxol. In such an embodiment of the invention, the liposomes may actually be formed in the body of the animal. Such animals are typically mammals, and preferably, are humans. Administration, which may be by any means known for the introduction of pharmaceutical substances comprising gels to the circulation of an animal, is preferably intravenous administration.

Still further provided is a process of preparing a liposome comprising taxol and a lipid which comprises the steps of: dispersing a mixture comprising taxol and the lipid in an organic solvent; contacting the resulting dispersion with an aqueous medium; concurrently emulsifying the aqueous medium while evaporating the organic solvent, so as to form a liposome comprising a plurality of lipid bilayers each of which encloses an aqueous compartment, wherein each of the aqueous compartments comprise a solute and wherein the concentration of the solute in each of the aqueous compartments is substantially equal; and, passing the liposome under pressure through a filter comprising pores of a defined size so as to reduce the lamellarity of the liposome, wherein the ratio of taxol to lipid (w/w) in the liposome is typically from about 0.01:1 to about 0.10:1, desirably about 0.03:1. However, the ratio may be higher or lower as needed. The lower limits will be governed by the least amount of taxol it is practical to make liposomes with, which may be readily determined by ordinarily skilled artisans. The upper limits will be governed by the concentration at which taxol crystallizes, i.e., the concentration at which it separates out from lipid bilayers and forms aggregates. (see Lenk et al., U.S. Patent Nos. 4,522,803, 5,030,453 and 5,169,637, Cullis et al., U.S. Patent No. 5,008,050 and Loughrey et al. U.S. Patent No. 5,059,421, the contents of which are incorporated herein by reference). Liposomes produced by this process are preferably unilamellar. Filters used to produce the liposomes preferably have defined pore sizes of about 100 nm or about 200 nm. The liposomes, LUVET<sub>100s</sub> or LUVET<sub>200s</sub>, also provided. Such liposomes, as well as the other liposomes provided herein, may be dehydrated according to the procedure of Janoff et al. (U.S. Patent No. 4,880,635, the contents of which are incorporated herein by reference). Dehydrated liposomes may be stored for extended periods of time without losing a substantial portion of their internal contents.

Pharmaceutical compositions provided herein will be useful for treating animals afflicted with a cancer. Such compositions comprise a pharmaceutically acceptable carrier and a liposome comprising taxol provided herein. For the purposes of this invention, a "pharmaceutically acceptable carrier" means any of the standard carriers, diluents, excipients and the like generally intended for use in connection with the administration of biologically active agents to animals. Such carriers are well known in the art and are generally chosen with regards to a number of factors, such as the particular drug being used and the intended route of administration, which are understood by the ordinarily skilled artisan. Pharmaceutical carriers preferred for use in accordance with the practice of this invention are those well known carriers suitable for use in connection with intravenous administration of liposomes and include, but are not limited to, sterile aqueous solutions such as physiological saline, 5% dextrose USP solutions and various aqueous buffers, e.g., aqueous phosphate buffers. The total solute concentration in such carriers should be controlled to keep the composition isotonic. Pharmaceutically acceptable carriers may also contain additional components, such as anti-oxidants, preservatives and the like, which are compatible with the active agent. The choice of such additional components is well within the purview of the ordinarily skilled artisan. Other carriers, e.g., tablets for oral administration and oils for mucosal or topical administration, may be prepared employing general knowledge and used in accordance with the practice of this invention.

Cancers which may be treated with the liposomes of this invention include, but are not limited to: brain, breast, ovarian, lung, and colon cancers or malignant melanomas. Taxol, because of its antimitotic activity and its ability to inhibit cell migration, is a valuable treatment for tumors, which exhibit more rapid cell division than normal tissues. Taxol's activity against ovarian, lung, brain, breast and other types of tumors has been well documented (see, e.g., Borman, Chemical and Engineering News, September 2, 1991; Edgerton, Biotechnology, 9: 933 (1991); The Pharmacological Basis of Therapeutics (Goodman Gilman et al., eds.), Pergamon Press, New York (1990); Rizzo et al., J. Pharm. & Biomed. Anal. 8(2):159 (1990); Donehower et al., Cancer Treat. Rep. 71(12):1171 (1987) Grem et al., Cancer Treat. Rep. 71(12):1179; Wiernik et al., J. Clin. Oncol. 5(8):1232 (1987), the contents of which are incorporated

herein by reference). In a presently preferred embodiment of this invention, the liposomal taxol formulations provided herein are used in the treatment of animals afflicted with ovarian cancer.

5           This invention further provides a unit dosage form of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective anticancer amount of a liposome comprising taxol provided herein. For the purposes of this invention, an "effective anticancer amount" is any amount of the liposome effective to treat a cancer, e.g., by inhibiting the growth of tumors or  
10 proliferation or metastasis of cancer cells. This amount will generally depend upon specific factors relevant to individual cases. Such factors are well known to ordinarily skilled artisans, or may readily be determined by them without undue experimentation. They include, but are not limited to: the type of cancer being treated and the stage of its progression; the type of animal being treated,  
15 as well as its age, weight and general condition; the particular drug being used, and whether it is being used in combination with other drugs; the type of liposome employed and the drug-to-lipid ratio in the liposome. As indicated herein, the ratio of taxol to lipids (weight/weight) in the liposomes of this invention is typically from about 0.01:1 to about 0.10:1, desirably about 0.03:1.  
20 However, the ratio may be higher or lower as needed. The lower limits will be governed by the least amount of taxol it is practical to make liposomes with, and may be readily determined by ordinarily skilled artisans. The upper limits will be governed by the concentration at which taxol crystallizes, i.e., the concentration at which it separates out from lipid bilayers and forms aggregates.  
25 Typically, the anticancer effective amount of a liposome is from about 0.1 mg of the liposome per kg of the body weight of an animal to which the pharmaceutical composition is administered to about 100 mg per kg of body weight; desirably, from about 1 mg per kg of body weight to about 50 mg per kg.

30           Further provided is a method of treating an animal afflicted with a cancer which comprises administering to the animal an amount of a gel comprising taxol and a lipid provided herein effective to treat the cancer by controlling or preventing the growth of tumors or the proliferation or metastasis of cancer cells. The cancers which may be so treated include, but are not limited  
35 to, brain, breast, lung colon, malignant melanoma or ovarian, with ovarian

cancer presently being the preferred object of treatment with the gels of this invention.

Gels are the solid or semisolid phases of colloidal solutions, in the case of this invention, of solutions of taxol and a lipid. A gel comprising a lipid may be solid lipid or a "soft" gel comprising lipid and an aqueous solution. Solid gels may be administered in accordance with the practice of this invention by any of the generally accepted means for doing so including, but are not limited to, implantation in tissues. However, the gels of this invention are preferably "soft" gels, i.e., gels comprising a lipid, taxol and an aqueous phase. The aqueous phases used will be suitable for administration to animals for the treatment of cancers. Accordingly, the preferred gels are pharmaceutical compositions comprising a lipid, taxol and a pharmaceutically acceptable carrier. Carriers suitable for use in gels and for administration to animals include, but are not limited to, aqueous buffered solutions such as phosphate buffered saline. Other components, such as antioxidants and preservatives, as well as additional aqueous buffers, may be used as needed. The gel may be administered to an animal such that liposomes are formed from the gel in the animal's body.

The gel may be provided in bulk, that is, in quantities larger than required for a single administration to an animal. The gel may also be provided as a unit dosage form, that is, in the amount needed to be administered in a single dose to an animal. Such a unit dosage form will comprise an effective anticancer amount of the gel. For the purposes of this invention, an "effective anticancer amount" of a gel is any amount of the gel effective to treat a cancer, e.g., by inhibiting the growth of tumors or proliferation of cancer cells. This amount will generally depend upon specific factors relevant to individual cases. Such factors are well known to ordinarily skilled artisans, or may readily be determined by them without undue experimentation. They include, but are not limited to: the type of disease being treated and the stage of its progression; the type of animal being treated, as well as its age, weight and general condition; the particular drug being used, and whether it is being used in combination with other drugs; and the relative proportions of taxol, lipid and aqueous phase in the gel. As indicated herein, the ratio of taxol to lipids (weight/weight) in the gels of this invention is typically from about 0.01:1 to about 0.10:1, desirably about 0.03:1. However, the ratio may be higher or lower as needed. The lower limits

will be governed by the least amount of taxol it is practical to make gels with, and may be readily determined by ordinarily skilled artisans. The upper limits will be governed by the concentration at which taxol crystallizes, i.e., the concentration at which it separates out from gels and forms aggregates.

- 5 Typically, the anticancer effective amount of a gel is from about 0.1 mg of the gel per kg of the body weight of an animal to which the gel is administered to about 100 mg per kg of body weight; desirably, from about 1 mg per kg of body weight to about 50 mg per kg.

- 10 This invention will be better understood from the examples which follow. However, those of ordinary skill in the art will readily understand that these examples are merely illustrative of the invention as defined in the claims which follow thereafter.

15

#### Examples

##### Example 1

- 20 Preparation of Monooleoyl Phosphatidylethanolamine Liposomes and Gels Containing Taxol

- Mixtures of taxol (0.6 mg in methylene chloride) and monooleoyl phosphatidylethanolamine (MOPE; 20 mg in methylene chloride) were dissolved  
25 in chloroform or methylene chloride. The organic solvents were then evaporated to form thin taxol/MOPE films. These films were hydrated with sodium carbonate buffer (10 mM) at a pH of about 7.5 or at a pH of about 9. MOPE has been shown to have detergent-like properties at pH of about 9, but to form bilayers at pH of about 7.5 (Tilcock et al. Biochem. 25: 816 (1986)). Light  
30 microscopy and freeze-fracture electron microscopy showed that liposomes were formed in the MOPE/taxol sample hydrated with the pH 7.5 solution. No taxol crystallization was observed. When the pH of the buffer surrounding the liposomes was increased to about pH 9, gels were formed.

- 35 Gels were formed from the MOPE/taxol films rehydrated with the pH 9 solution. There was a small amount of aqueous phase above this gel which did



not contain taxol. Dilution of the gel ten-fold with buffer resulted in the formation of a clear, viscous solution, in which no crystallization of taxol was observed.

5 Example 2

Formation of Egg Phosphatidylcholine Multilamellar Liposomes (MLVs) Containing Taxol

10 Solutions of egg phosphatidylcholine (EPC) and taxol were formed by dissolving EPC/taxol mixtures (100 mg EPC and 3 mg taxol) in chloroform or methylene chloride. Addition of sodium carbonate buffer resulted in the formation of multilamellar liposomes. No crystallization of taxol was observed either by light or electron microscopy and no taxol pellet was seen upon  
15 centrifugation in histopaque. However, small rectangular taxol crystals, which precipitate upon centrifugation in histopaque (50% dilution), were observed when these multilamellar liposomes were sonicated.

Example 3

20

Formation of Egg Phosphatidylcholine/Taxol Liposomes Having Substantially Equal Interlamellar Solute Distribution

Liposomes were formed by dissolving a mixture of 100 mg of EPC and 3  
25 mg of taxol in 1.5 ml methylene chloride, adding 5 ml of sodium carbonate buffer and emulsifying the buffer while evaporating the methylene chloride, with a nitrogen stream, according to the procedure described in Lenk et al. (U.S. Patent Nos. 4,522,803, 5,030,453 and 5,169,637). No crystallization of taxol was observed in these formulations.

30

#### Example 4

##### Formation of Egg Phosphatidylcholine/Taxol Liposomes Having Substantially Equal Interlamellar Solute Distribution

5

Liposomes containing EPC, but no taxol (placebo vesicles), were formed with 10 grams of EPC and 75 ml of methylene chloride according to the above-described procedure. The EPC and methylene chloride were mixed with an A-200 propeller (high shear). The mean diameter of the liposomes formed, as  
10 determined by NICOMP (see Figure 1), was 204.5 nm. Freeze-fracture electron microscopy showed liposomes with diameters of from about 65 nm to about 350 nm, in agreement with the NICOMP measurements.

Liposomes containing EPC and taxol were formed using 10 grams of  
15 EPC, 300 mg of taxol and 75 ml of methylene chloride by the same procedure. The mean diameter of the liposomes formed, as determined by NICOMP, was 309.8 nm (see Figure 2). Freeze-fracture electron microscopy showed that vesicles with diameters of from about 90 to about 1200 nm were formed. The EM studies also showed that the interlamellar spacings in these liposomes were  
20 large and irregular, which is indicative of a repulsion between the layers. No taxol crystallization was observed by light or EM microscopy or upon centrifugation in histopaque. Crystals 5 to 15 microns long were observed, by light microscopy, after the liposomes had been stored for about one week in the cold room (4 deg. C.) These were removed by centrifugation in histopaque and  
25 removing the resulting pellets. Heating did not result in disappearance of the crystals, but freeze-thawing did not generate any new crystals.

#### Example 5

30

##### Preparation of EPC/Taxol Unilamellar Liposomes

Multilamellar liposomes containing EPC and taxol (MW 853.9) were prepared as described above (see Example 3). The MLVs were then extruded  
35 through 0.1 micron and 0.2 micron Nucleopore™ filters. Taxol concentrations were determined by spectrophotometry using an extinction coefficient of  $e =$

29,700 L/mol·cm<sup>-1</sup> at 229 nm. Due to the overlap with the EPC peak, subtraction of this peak was performed by using an equivalent amount of EPC in the reference cell. Five percent of the taxol contained within the EPC MLVs was lost after the multiple extrusions through the 0.2 micron filters. Nine  
5 percent was lost after extrusion through the 0.1 micron filters. No taxol crystallization was observed in any of the unilamellar liposomes produced by the extrusion process.

What is claimed is:

1. A liposome having a bilayer which comprises taxol and a lipid comprising a lysophospholipid, wherein the ratio of taxol to lipid (w/w) is from about 0.01: 1 to about 0.10:1.
2. The liposome of claim 1, wherein the liposome is a unilamellar liposome.
3. The liposome of claim 2, wherein the unilamellar liposome is a large unilamellar liposome.
4. The liposome of claim 1, wherein the liposome comprises a plurality of lipid bilayers each of which encloses an aqueous compartment.
5. The liposome of claim 4, wherein each of the aqueous compartments comprises a solute and the concentration of the solute in each of the aqueous compartments is substantially equal.
6. The liposome of claim 1, wherein the lysophospholipid is a lysophosphatidylethanolamine.
7. The liposome of claim 6, wherein the lysophosphatidylethanolamine is monooleoyl phosphatidylethanolamine.
8. The liposome of claim 1, wherein the lipid further comprises a sterol.
9. The liposome of claim 8, wherein the sterol compound comprises cholesterol.
10. A process of preparing a liposome having a bilayer which comprises taxol and a lipid comprising a lysophospholipid which comprises the steps of:
  - (a) mixing a film comprising taxol and the lipid; and

(b) contacting the resulting mixture with an aqueous medium having a pH effective to form a liposome such that the aqueous medium is both internal and external to the resulting liposome,

5 and wherein the ratio of taxol to lipid (w/w) in the liposome is from about 0.01:1 to at least about 0.10:1.

11. The process of claim 10, wherein the ratio of taxol to lipid (w/w) in the liposome is about 0.03:1.

10

12. The process of claim 10, wherein the lysophospholipid is a lysophosphatidylethanolamine.

13. The process of claim 12, wherein the lysophosphatidylethanolamine is monooleoyl phosphatidylethanolamine.

15

14. The liposome prepared by the process of claim 10.

15. The process of claim 10, comprising the additional step of increasing the pH of the aqueous medium external to the liposome to a pH effective to form a gel.

20

16. The gel formed by the process of claim 15.

25 17. A process of preparing a gel which comprises taxol and a lipid comprising a lysophospholipid which comprises the steps of:

(a) mixing taxol and the lipid; and

30 (b) contacting the resulting mixture with an aqueous medium having a pH effective to form a gel,

wherein the ratio of taxol to lipid (w/w) in the gel is from about 0.01:1 to at least about 0.10:1.

35 18. The process of claim 17, wherein the lysophospholipid is a lysophosphatidylethanolamine.

19. The process of claim 18, wherein the lysophosphatidylethanolamine is monooleoyl phosphatidylethanolamine.
- 5 20. The gel prepared by the process of claim 17.
21. The process of claim 17, comprising the additional step of decreasing the pH of the aqueous medium surrounding the gel to a pH effective to form liposomes.
- 10 22. The process of claim 21, wherein the pH is decreased by administering the liposomes to the circulatory system of an animal.
23. The liposomes prepared by the process of claim 21.
- 15 24. A process of preparing a liposome comprising taxol and a lipid which comprises the steps of:
- 20 (a) dispersing a mixture comprising taxol and the lipid in an organic solvent;
- (b) contacting the resulting dispersion with an aqueous medium;
- 25 (c) concurrently emulsifying the aqueous medium while evaporating the organic solvent, so as to form a liposome comprising a plurality of lipid bilayers each of which encloses an aqueous compartment, wherein each of the aqueous compartments comprises a solute and the concentration of the solute in each of the aqueous compartments is substantially equal; and
- 30 (d) passing the liposome under pressure through a filter comprising pores of a defined size so as to reduce the lamellarity of the liposome,
- 35 wherein the ratio of taxol to lipid (w/w) in the liposome is from about 0.01:1 to about 0.10:1.

25. The process of claim 24, wherein the liposome is a unilamellar liposome.
26. The process of claim 24, wherein the pore size of the filter is about 200 nm.
- 5 27. The process of claim 24, wherein the pore size of the filter is about 100 nm.
- 28 The liposome prepared by the process of claim 24.
- 10 29. The liposome of claim 1, 14, 23 or 28, wherein the liposome is dehydrated.
30. A pharmaceutical composition comprising the liposome of claim 1, 14, 23  
15 or 28 and a pharmaceutically acceptable carrier.
31. A method of treating an animal afflicted with a cancer which comprises administering to the animal an amount of the pharmaceutical composition of claim 30 effective to treat the cancer.
- 20 32. The method of claim 31, wherein the animal is a human and the cancer is ovarian cancer.
33. A unit dosage form of a pharmaceutical composition comprising a  
25 pharmaceutically acceptable carrier and an effective anticancer amount of the liposome of claim 1, 14, 23 or 28.
34. The unit dosage form of claim 33, wherein the anticancer effective amount of the liposome is from about 0.1 mg of the liposome per kg of the  
30 body weight of an animal to which the pharmaceutical composition is administered to about 100 mg per kg of body weight.
35. A method of treating an animal afflicted with a cancer which comprises administering to the animal an amount of the gel of claim 16 or 20  
35 effective to treat the cancer.

36. A unit dosage form of the gel of claim 16 or 20 which comprises an amount of the gel effective to treat a cancer in an animal.
- 5 37. The unit dosage form of claim 36, wherein the amount of the gel effective to treat the cancer is from about 0.1 mg of the gel per kg of body weight of an animal to which the gel is administered to about 100 mg of the gel per kg of the animal's body weight.



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## Gaussian Analysis (Vesicles)

placebo for taxol, 9/11/91

| SIZE nanometers | REL. VOLUME |
|-----------------|-------------|
| 34.6            | 0.4         |
| 40.8            | 1.5         |
| 47.8            | 4.8         |
| 55.1            | 12.6        |
| 63.7            | 27.7        |
| 77.0            | 50.3        |
| 90.3            | 75.4        |
| 101             | 95.0        |
| 107             | 100.0       |
| 124             | 85.0        |
| 145             | 52.0        |
| 170             | 36.3        |
| 195             | 15.7        |
| 234             | 7.7         |
| 274             | 2.1         |
| 321             | 0.7         |
| 357             |             |
| 442             |             |
| 518             |             |
| 607             |             |
| 711             |             |
| 834             |             |
| 978             |             |

FIGURE 1

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## Gaussian Analysis (Vesicles)

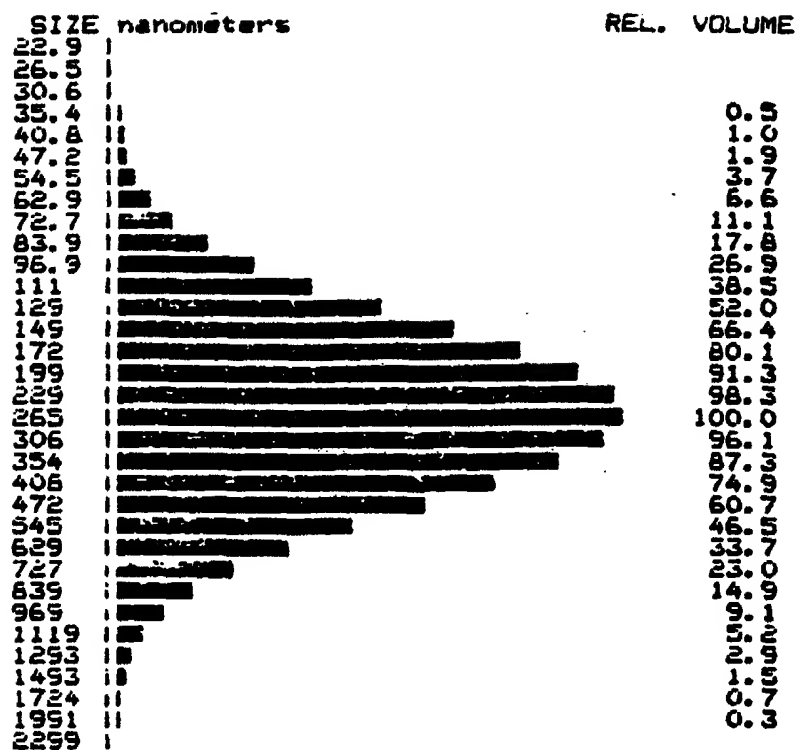


FIGURE 2

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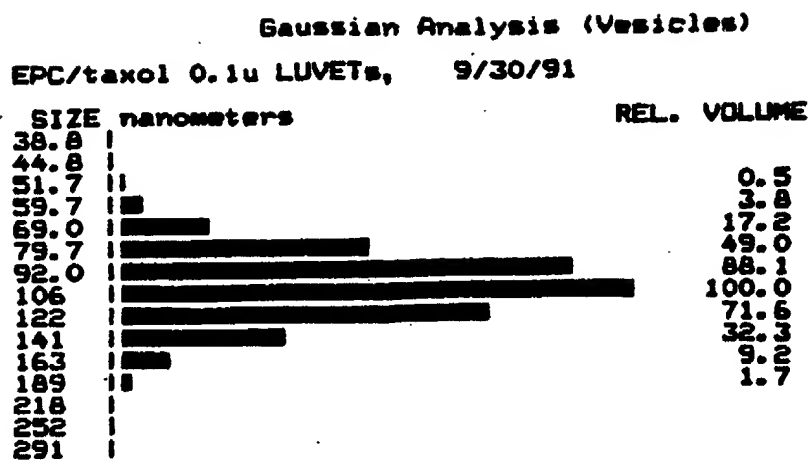


FIGURE 3

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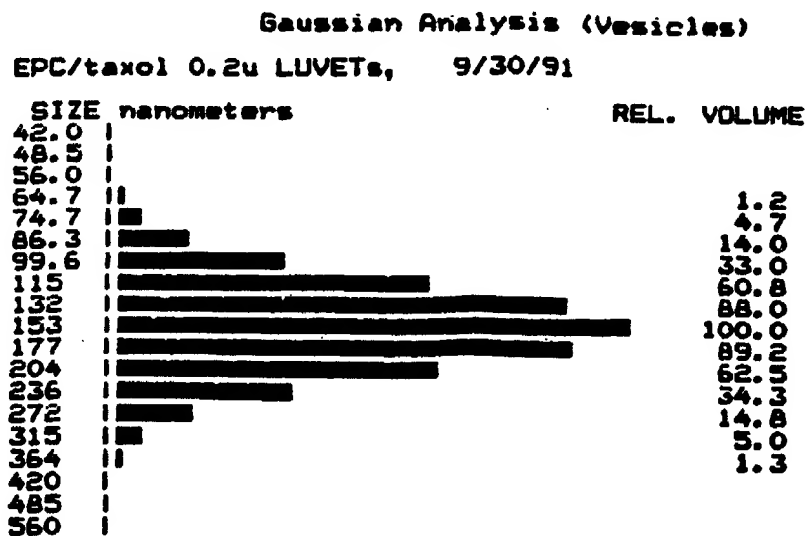


FIGURE 4

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 94/05386

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 5 A61K9/127 A61K47/24 A61K31/335

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|------------|--|-----------------------|
| X          | J. MICROENCAPSULATION,<br>vol.7, no.2, May 1990, LONDON (GB)<br>pages 191 - 197<br>M.-H. BARTOLI ET AL. 'in vitro and in vivo<br>antitumoral activity of free and<br>encapsulated taxol' | 1-14,<br>24-34        |
| Y          | see the whole document<br><br>see page 192   | 15-23,<br>35-37       |
| Y          | WO,A,90 09385 (THE LIPOSOME COMPANY) 23<br>August 1990<br>see the whole document   | 15-23,<br>35-37       |
| A          | EP,A,0 118 316 (LIPID SPECIALITIES, INC.)<br>12 September 1984<br>see page 7, line 20 - page 8, line 33<br>see page 14; example 10   | 1-37                  |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance  
 "B" earlier document but published on or after the international filing date  
 "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)  
 "O" document referring to an oral disclosure, use, exhibition or other means  
 "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  
 "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone  
 "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.  
 "&" document member of the same patent family

Date of the actual completion of the international search

30 August 1994

Date of mailing of the international search report

06.09.94

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## INTERNATIONAL SEARCH REPORT

international application No.

PCT/US 94/05386

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
**REMARK: ALTHOUGH CLAIMS 31, 32, 35 ARE DIRECTED TO A METHOD OF TREATMENT OF THE HUMAN/ANIMAL BODY THE SEARCH HAS BEEN CARRIED OUT AND BASED ON THE ALLEGED EFFECTS OF THE COMPOSITION.**
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. al Application No

PCT/US 94/05386

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|---|---------------------|----------------------------|---------------------|
| WO-A-9009385                              | 23-08-90            | AU-B- 639228               | 22-07-93            |
|   |                     | AU-A- 5173690              | 05-09-90            |
|   |                     | EP-A- 0458894              | 04-12-91            |
|   |                     | JP-T- 5500203              | 21-01-93            |
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|   |                     | JP-A- 59204198             | 19-11-84            |
|   |                     | US-A- 4507217              | 26-03-85            |
| -----                                     |                     |                            |                     |